in expression of these genes was a higher level of IL-8 in males compared to females for MT ($p < 0.0001$) and MT+ACL tear ($p < 0.05$).

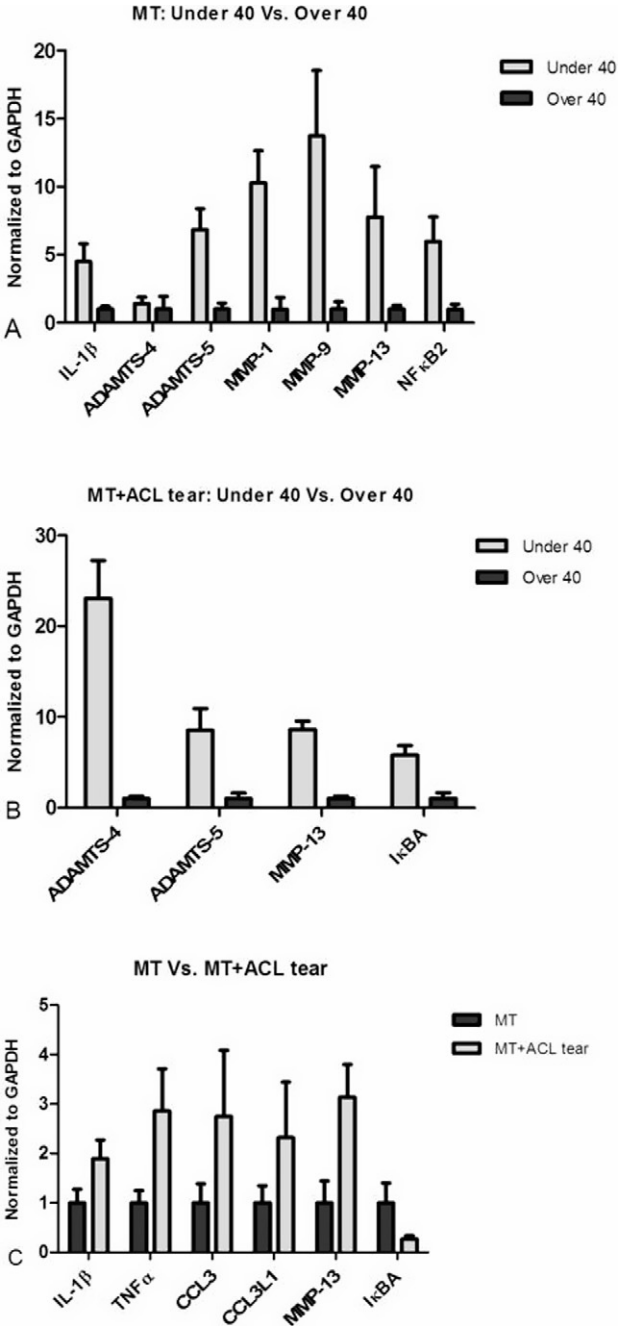

Fig. 1. Gene expression pattern in age-related meniscal tears with and without concomitant ACL tear. All results presented here are statistically significant ($p < 0.05$) within each graph between the two categories.

Conclusions: Gene expression in meniscal tears varies by patient age, sex and injury pattern. Our findings suggest that elevated expression levels of OA-specific markers indicate an increased catabolic (inflammatory) response in young patients with MT as well as MT+ACL tear. Furthermore, higher expression of inflammatory markers in MT+ACL tear compared to MT alone provide molecular evidence suggesting that the combined injury pattern is more likely to lead to the development of OA. These findings suggest clinically relevant differences in the response of the knee to meniscus and ACL tears based on patient age and sex. Catabolic activity may be predictive of patients at risk for progression of OA following partial meniscectomy and ACL reconstruction.

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CALCIUM DEPOSITION AND GLYCOSAMINOGLYCAN PRESENCE IN OSTEOARTHRITIC HUMAN MENISCAL ATTACHMENTS

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Purpose: Meniscal attachments are unique graded tissue interfaces that diffuse hoop stress from the meniscal body to the tibial plateau. Identified enthesopathic alterations at other tissue interfaces have shown, amongst other changes, an increase in water-affine proteoglycan content, which may result in extracellular matrix swelling and disruption of the fiber network. If the attachments become structurally compromised, excessive transverse meniscal extrusion results, and such extrusion is a known precursor to knee osteoarthritis. Coupling this information with noted catabolic activity within the arthritic joint gives rise to the supposition that the meniscus-to-bone interface is a potential disease-forming pathway, possibly predating other harbingers of degradation. To date there have been no studies examining bio-chemical changes at this interface.

Methods: Tibial plateaus from healthy ($n = 4$) cadavers and patients with end-stage knee osteoarthritis ($n = 3$) (undergoing total knee arthroplasty) were obtained with IRB approval. All four (medial anterior, lateral anterior, medial posterior, and lateral posterior) meniscal attachments from ligamentous zone to subchondral bone were excised, fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, and embedded in glycol methacrylate. Specimens were sectioned using a diamond wafering blade, ground using progressive SiC sheets on an autopolishing wheel and cleared in xylene. Sections were stained using the Von Kossa (VK) technique for calcium deposits and counterstained using toluidine blue (TB) to identify glycosaminoglycans (GAG).

Changes in GAG presence in the insertion zones were quantified using Bioquant OSTEO software to identify the thickness of the TB stained and the TB+VK stained regions (Figure 1). Each region was outlined and measurements were performed at 20 μm intervals. Calcium deposition was scored using a modified grading scale as described by Sun et al. 2010 (Table 1). Two blind reviewers examined each section independently and results were averaged between them.

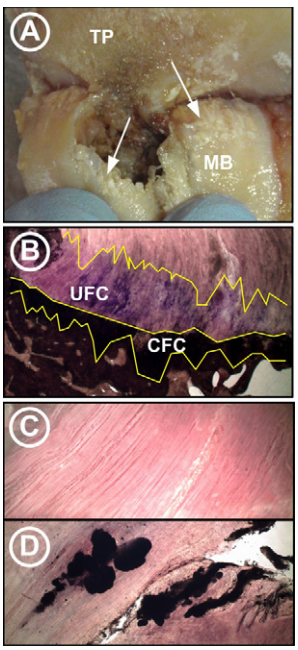

Fig. 1. (a) Gross inspection of insertion site reveals apparent calcium deposition on the surface. TP – Tibeal Plateau, MB – Meniscal Body. (b) Meniscal insertion stained for GAG and calcium. Yellow lines show the regional identification used for thickness measurements. AC – Articular Cartilage, UFC – Uncalcified FibroCartilage, CFC – Calcified FibroCartilage. Color thresholding (not pictured) used to aid in regional identification. (c) Fibrous attachment with no calcium deposition. (d) Calcium deposits within the fibrous attachment (left) and articular cartilage (right).

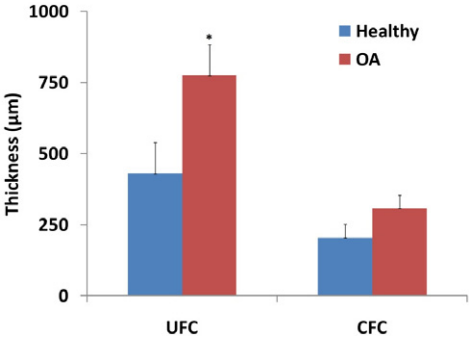

Fig. 2. GAG Thickness measurements in the meniscal attachment. Significant differences were observed between the healthy and OA samples in the uncalcified fribocartilage thickness (*P < 0.05).

Table: Calcium deposit ranking scale, modified from Sun et al. 2010

Score	Description
0	No calcium presence
1	Small calcium deposits around the exterior
2	Large deposits around the exterior
3	Calcium deposits around exterior and small deposits within the fibrous attachment
4	Calcium deposits around exterior and large deposits within the fibrous attachment

A two-way ANOVA considering attachment health and location was used to examine differences in GAG thickness. A Wilcoxon rank sum test was used to elucidate differences in calcium deposition between the healthy and arthritic group of attachments.

Results: Significant differences in measured GAG thickness between the healthy and osteoarthritic attachments were observed (Figure 2), however no differences were observed between attachment sites. The calcium deposition of the OA samples demonstrated severe aggregation of calcium within and around the fibrous attachment. Accordingly, calcium deposition was significantly differently between groups using the scoring system described ($p < 0.01$).

Conclusions: These results coincide with other pathological tissue studies, revealing a localized increase in GAG thickness at the insertion site as well as distinct calcium deposition in arthritic specimens (Figure 1). This continues to build on the theory that calcium deposition, while not necessarily causative, is an obvious result of OA. It is apparent that proposed anti-calcification treatments would need to consider the entire meniscus and its attachments as an entire functional unit. Additionally, work within our laboratory has identified an increased stiffness disparity at the interface at the interface using nano-indentation. Future work will include performing calcium assays to quantify the amount of deposition and also incorporating FE modeling to elucidate the impact the calcium deposits and changes in mechanical properties have on the integrity of the attachments.

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ANGIOGENESIS-RELATED CYTOKINE PROFILES IN PATIENTS WITH ROTATOR CUFF DISEASE: HOW EFFECTIVE ARE SEROLOGICAL MARKERS FOR EARLY DIAGNOSIS?

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Introduction: Approximately 16% of the general population is believed to have rotator cuff disease (RCD) at a given time. The hallmarks of RCD the pathogenesis are inflammation, abnormal immune response, angiogenesis and variables of vascularity. Avascular zones of tendons are predisposed for degenerative changes and spontaneous rupture in RCD. Decreased and increased vascularity might be involved in the pathogenesis of degenerative tendon disease. In healthy individuals, the cytokines synthesis of tendon increases in response to physical activity, which results in stronger skeletal muscle and more resistant connective tissue. Abnormalities in angiogenesis-related cytokines (ARC) network of importance for the clinical signs and symptoms will be focused on.

Objectives: To evaluate the diagnostic performance of serological tests for early degenerative changes in RCD patients: the alterations in endogenous ARC expression (IL-1beta, IL-6, IL-8, IL-10, VEGF, bFGF, ANG) in the peripheral blood sera.

Methods: It is a prospective study of 200 patients with RCD (mean age 66.5). Patients were followed with clinical measures (UCLA, Constant, WORC). MRI studies were performed on each shoulder. The control group consisted of 200 age-, sex-matched healthy controls. Angiogenesis imaging assays was performed using PDUS for evaluation the variables of vascularity of the rotator cuff tendons. ARC profiles was performed using Immunoassay kits (Bio-Plex Human Cytokine Assay; Bio-Rad Inc., Hercules, CA, USA) per the manufacturer's instructions, NIBSC's International Standards and ECBS's recommendations of the World Health Organization for of cytokine measurements.

Results: At time of inclusion the concentration of IL-1beta, IL-8, VEGF was significantly higher in RCD patients than in control. Serum VEGF levels significantly higher was found in 85% RCD patients studied. The overexpression of VEGF correlated with advanced stage ($r = 0.75$; $p < 0.0005$), aMVD ($r = 0.68$, $p < 0.005$) and VAS ($r = 0.75$, $p < 0.0002$) in RCD patients. Serum ANG and IL-10 levels significantly lower in RCD patients versus control. Levels IL-1beta and ANG significantly correlate with degenerative tendon grade in RCD patients. No difference in IL-6 and bFGF levels between RCD patients and control. Patients with degenerative changes had very lower serum ANG levels compared with controls. Combination of high VEGF and low ANG concentrations in the sera have been considered as a predisposing factor for patients with ruptures of rotator cuff tendons. PDUS examination revealed a high blood vessel density in patients with tendon ruptures.

Conclusions: This investigation provides the first standardized analytic approach for assessing differences in ARC levels in the sera of RCD patients. Our data demonstrated that uncontrolled pathogenesis of RCD is associated with an imbalance between inflammatory, antiinflammatory and vascular ARC. The future of ARC in clinical RCD application requires close interactions between the public and private sectors: the government, the pharmaceutical and biotechnology industries, and academia.

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ANALYSIS OF MENISCUS USING FOURIER TRANSFORM INFRARED IMAGING SPECTROSCOPY

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Purpose: Fourier Transform Infrared Imaging Spectroscopy (FT-IRIS) has been used to evaluate compositional and structural differences of extracellular matrix (ECM) of cartilage. However, ECM of meniscus has not been analyzed by FT-IRIS. Meniscus can be divided into two portions, the central portion and the peripheral portion. The central portion is designated the "white zone" (WZ), which is an avascular zone. The peripheral portion is designated the "red zone" (RZ), which is a vascular zone. The purpose of this study was to analyze differences of collagen and proteoglycan in the extracellular composition of the WZ and the RZ of meniscus using FT-IRIS analysis and biochemical assays.

Methods: Six menisci were removed from six porcine knees. The WZ and the RZ of the menisci were analyzed using FT-IRIS analysis and biochemical assays. In FT-IRIS analysis, the integrated area of amide I, 1710–1590 cm-1, for collagen and the area of sugar peak, 1150–950 cm-1, for proteoglycan were calculated using the Spectra Manager ver. 2 software (Jasco Co. Japan). In biochemical assays, the WZ and the RZ of the menisci were digested with RIPA buffer. Aliquots of the digests were assayed separately with hydroxyproline content for collagen and with dimethylmethylene blue (DMMB) for proteoglycan by quantifying the amount of sulfated glycosaminoglycans. Mann-Whitney test was used for statistical analysis. Correlation between the FT-IRIS data and the biochemical data was evaluated with Spearman's rank correlation coefficient. A P value <0.05 was considered to be significant.

Results: According to the amide I area of FT-IRIS, collagen in WZ (38.8 ± 12.2 arbitrary units) was significantly lower than that in RZ (59.6 ± 9.1 arbitrary units; $P = 0.004$). Hydroxyproline content in WZ ($2.65 \pm 1.95 \times 10^4$ μg/mL/cm^3) was significantly lower than that in RZ ($5.95 \pm 1.62 \times 10^4$ μg/mL/cm^3; $P = 0.024$). There was a significant correlation ($R2 = 0.72$) between the collagen estimated by amide I area of FT-IRIS and the hydroxyproline content. According to the sugar area of FT-IRIS, proteoglycan in WZ (5.5 ± 1.71 arbitrary units) was significantly higher than that in RZ (3.38 ± 2.24 arbitrary units; $P = 0.03$). Proteoglycan